REMARKS

Claims 83 was pending in this application, and was rejected. Claim 83 has been amended to recite that the protein encoded by the transcript variant is immunoreactive with at least one antibody that specifically binds the amino acid sequence of SEQ ID NO: 2. Support for amended claim 83 can be found throughout the original application and *inter alia*, at least at page 31, paragraph **[0138]** of the substitute specification submitted April 4, 2007.

With respect to all amendments, Applicants have not dedicated or abandoned any unclaimed subject matter and moreover have not acquiesced to any rejections and/or objections made by the Patent Office. Applicants expressly reserve the right to pursue prosecution of any presently excluded subject matter or claim embodiments in one or more future continuation and/or divisional application(s).

Reconsideration of the claim in view of the following comments is respectfully requested. Applicants have carefully considered the points raised in the Final Office Action and believe that the Examiner's concerns have been addressed as described herein, thereby placing this case into condition for allowance.

Maintained Rejection

Claim 83 currently stands rejected only for lack of enablement.

Rejection Under 35 U.S.C. § 112, First Paragraph, Enablement

Claim 83 was rejected for lack of enablement. The Office maintained that the specification, while being enabling for an isolated 121P1F1 transcript that encode the protein of SEQ ID NO:2, does not reasonably provide enablement for any isolated transcript variants that encodes a protein comprising at least one amino acid substitution, addition or deletion relative to SEQ ID NO:2 or the transcript variant of SEQ ID NO:5. The Examiner is essentially asserting that there is no objective evidence that the transcript variant specified in the claim possess the same

properties as the generic 121P1F1 sequence, such that the transcript variants are over-expressed in cancers.

Applicants disagree, and respectfully traverse the rejection.

Rather than revisit Applicants' previous arguments, which are incorporated by reference herein, Applicants note that the U.S. Patent and Trademark Office issued U.S. Patent No. No. 6,924,358 ("the '358 patent") and has allowed USSN 11/125,805, Notice of Allowance mailed 08/07/2007. USSN 11/125,805 is a continuation of the '358 patent. The present application claims priority to the '358 patent, and thus is related to both the '358 patent and USSN 11/125,805. The claims of the '358 patent are directed to an isolated protein encoded by SEQ ID NO:1 to the protein comprising SEQ ID NO: 2. The protein recited in the issued claims the '358 patent is useful as a diagnostic as well as a therapeutic target on cancer cells. The claims of USSN 11/125,805 are directed to an isolated nucleic acid encoding the amino acid sequence of SEQ ID NO:2, expression and viral vectors comprising the nucleic acid sequences, and host cells comprising the expression vector.

Allowance and issuance of the parent '358 case indicates that the protein sequence in question is presumed to possess the qualities of a useful and enabled invention. *See* 35 U.S.C. § 282, "A patent shall be presumed valid." Moreover, the Examiner has himself acknowledged that the specification is enabling for a transcript that encodes the protein of SEQ ID NO:2. (10.11.2007 AA at page 2). In view of the statutory imprimatur of validity for the protein of SEQ ID NO:2, it naturally flows that claims directed to protein variants that are immunoreactive with at least one antibody that specifically binds the amino acid sequence of SEQ ID NO: 2 enjoy the same patentable qualities of usefulness and enablement of the parent case. In the present case, the functional feature of the claimed protein is its ability to immunoreact with antibodies raised against SEQ ID NO: 2.

Lerner (Nature 299:592-596 (1982), enclosed herein as Exhibit A) is cited as indicating that antibody epitopes can be as small as 6 to 15 amino acid residues long. This is evidence against

the unpredictability of the protein of claim 1 (that is, Lerner's article supports the enablement of claim 83). As described above, claims directed to SEQ ID NO:2 were issued in U.S. Patent No. No. 6,924,358. Since a protein falling within claim 83 has several stretches of 6 to 15 amino acids in common with the protein encoded by SEQ ID NO: 2, such a protein is likely to share several immunogenic epitopes with the protein encoded by SEQ ID NO: 2. There is little "unpredictability," if any, that the proteins of claim 83 will work for the purpose of provoking an immune response.

In view of these amendments and comments, Applicants respectfully request that the Examiner withdraw the present rejection and pass the case to issuance.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

In the event the U.S. Patent and Trademark office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket **No. 511582003420**. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: October 30, 2007

Respectfully submitted,

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REVIEW ARTICLE

Tapping the immunological repertoire to produce antibodies of predetermined specificity

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We understand the structure of antibodies in detail, but know little about the molecular basis of the immunogenicity of proteins. Recent experiments have shown that chemically synthesized peptides representative of virtually any part of the surface of a protein can elicit antibodies reactive with the native molecule. Such peptides can serve as synthetic vaccines, and antibodies, useful in the study of changes in protein structure, can be generated. As these antibodies react with regions of the protein known in advance to the experimenter, they can be said to be of predetermined specificity.

THE immune system of a mammal is one of the most versatile systems in the biological kingdom as probably greater than 1.0×10^7 antibody specificities can be produced[1]. Indeed, much of contemporary biological and medical research is directed toward tapping this repertoire. As it is so vast, it might appear to be a relatively simple matter to produce antibodies of a particular specificity but until recently this was not the case because of two essential complications. The first problem is that serum antibodies consist of a mixture of molecules of diverse reactivity. As such they are useful for studying whole proteins but an understanding of fine specificity is difficult if not impossible. The development by Köhler and Milstein of the hybridoma methodology has solved this problem by making it possible to obtain in pure form antibodies of a single specificity from those induced during an immune response[2] but this left a second problem in that during an immune response to an intact protein antibodies are only produced against a very limited set of determinants within the protein molecule. This problem is the one to be discussed here.

Limited response to intact proteins

The key to eliciting antibodies of predetermined specificity is an understanding of what constitutes an immunogenic determinant on a protein. Whereas the term an antigenic determinant simply reflects the ability of a region in a protein to bind to antibodies, an immunogenic determinant is a region capable of inducing antibody. (Here we further define an immunogenic determinant as one that induces antibody reactive with the native protein.)

Previous studies on the nature of antigenic determinants, mostly following immunization with intact proteins, have led to two fundamental conclusions (reviewed in ref. 3, see also refs 4–21). The first is that during an immune response to a native protein, antibody reactivity is confined to only a few regions of the molecule. Studies on enzymatically fragmented proteins suggested that most globular proteins contain fewer than five antigenic sites; as a rough rule, about one site per 5,000 daltons of protein. The second conclusion is that antigenic determinants are dependent on tertiary conformation and are often constructed from discontinuous regions of the protein chain brought into proximity by folding of the molecule. These determinants are called 'conformational' or 'discontinuous'. Thus, by the mid-1970s, a picture of the antigenic profile of a protein had emerged. The determinants are few in number and largely dependent on native conformation (Fig. 1). However, throughout these studies it was tacitly assumed that antigenicity and immunogenicity are equivalent; in other words, the number of antigenic determinants of an intact protein was presumed to set a limit on the number of protein fragments which would carry immunogenic determinants.

The repertoire can be tapped

Needless to say, the above concepts did not bode well for a general method of producing antibodies reactive with most regions of a protein molecule. Our own interest in the problem followed an experiment we carried out to detect a protein potentially encoded by the Moloney leukaemia virus genome. During our sequencing of this genome, we found an open reading frame whose coding capacity did not fit with the known biochemistry of the viral proteins, a problem we have called genotype in search of phenotype. We decided to synthesize chemically a peptide from within the protein predicted by the nucleotide sequence, raise an antibody to it, and see if that antibody reacted with protein(s) in infected cells. Because of uncertainties as to how or if RNA splicing might be taking place, we synthesized a peptide potentially encoded by the 3′ end of the reading frame, and thus representative of the C-terminus of the putative protein. Indeed, the anti-peptide antibody precipitated a protein from infected but not normal cells[22]. Such an approach could be very useful as more and more DNA sequences were generated, but it was far from proven as a general methodology. We had studied the C-terminus of a protein and it seemed possible that the method might be useful only in detecting the ends of proteins. It was thought that the untethered C-terminus of a protein was relatively free to rotate and could be thought of as a kind of hapten carried by the rest of the molecule, a situation which we could have fortuitously duplicated when we coupled the peptide to the carrier protein for immunization.

To test the generality of the method we carried out a study on a protein of known structure. We chose the influenza virus haemagglutinin (HA) because the complete nucleic acid sequence of its gene is available[23] and its crystallographic structure is known at high resolution[24]. A series of peptides covering 75% of the HA1 chain were chemically synthesized (we have now synthesized additional peptides so that the entire span of the molecule is represented) and antibodies made to each of the peptides. Antibodies to almost all (18 of 20) peptides react with the native molecule[25]. Because in its folded state the HA1 molecule displays a number of conformations including α-helix, random coil and β-sheets, it is clear that the ability of the anti-peptide antibodies to react with the intact structure is independent of any particular conformation or location in the molecule[25]. Probably the only requirement for selecting an immunogenic peptide is that a part of the sequence be located on the surface of the molecule so as to be available to antibody. In Fig. 2*b*, we show the portion of the surface of the HA1 molecule against which we have made antibodies using chemically synthesized peptides as immunogens. In Fig. 2*a*, we show those areas of the molecule thought to be immunogenic during viral infection or immunization with intact virus or purified viral

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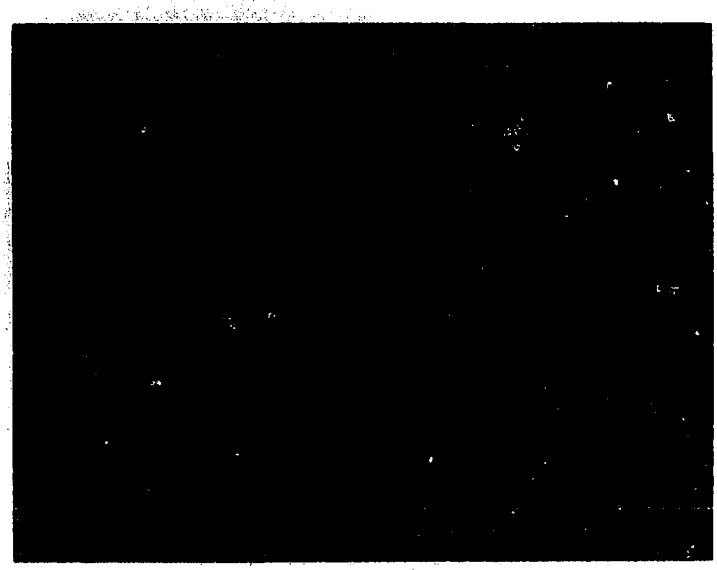
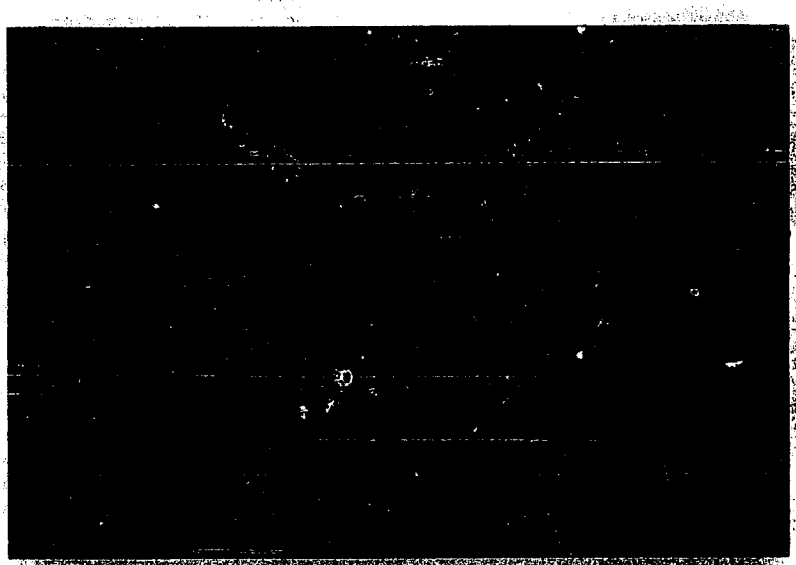


Fig. 1 Exposed surfaces of antigenic sites (shown in pink) of hen's egg-white lysozyme. Stereo projections showing the limited number of antigenic sites in the lysozyme molecule and their discontinuous ('conformational') nature. The data are based on Atassi and Lee[71] using the structure of Blake *et al.*[72] and Imoto *et al.*[73]. Exposed surfaces based on α-carbon positions using molecular surface computer program of Connolly[74]. The three antigenic sites are constructed by the spatially contiguous residues as follows: (1) Arg 125, Arg 5, Glu 7, Arg 14 and Lys 13; (2) Trp 62, Lys 97, Lys 96, Asn 93, Thr 89 and Asp 87; (3) Lys 116, Asn 113, Arg 114, Phe 34 and Lys 33.

a



b

Fig. 2 Antigenic and immunogenic sites (shown in pink) of the influenza virus HA1 molecule. (HA1 shown in green, HA2 shown in blue.) Structure of the molecule based on crystallographic coordinates of Wilson *et al.*[31]. Exposed surfaces based on α-carbon positions using molecular surface computer program of Connolly[74]. *a*, Stereo-pair representing sites eliciting antibodies during the process of infection or immunization with intact virus. *b*, Stereo-pair representing sites against which antibody can be induced by immunization with chemically synthesized peptides.

proteins[26-28]. The conclusions from these studies were clear—the immunogenicity of a protein is less than the sum of the immunogenicity of its pieces[25] with, however, one caveat. The map in Fig. 2 is based on neutralization data and it is possible that during ordinary immunization, antibodies reactive with other parts of the protein are generated but are not scored because they are not neutralizing. (This problem arises because not all antibodies that bind to viral proteins inhibit the infectivity of the virus.) If this were the case then the collection of anti-virus antibodies would have a reactivity pattern much broader than that observed by neutralization studies. This is, however, probably not the case: we have found that high-titred antibody made against the intact haemagglutinin does not react with any of the synthetic peptides[25]. We recently carried out a more compelling demonstration of the exclusion of some reactivities in anti-virus sera by taking advantage of the facts that among various influenza strains there are constant and variable regions of the HA1 and HA2 components of the viral haemagglutinin, and that antibody to the native molecule does not widely neutralize across strains. If an anti-peptide antibody to a conserved region neutralized across strains whereas an anti-virus antibody did not, it would indicate that different immunological specificities are generated during the two types of immunization. We therefore studied antibodies to several peptides from conserved portions of the protein structure and showed that even though the anti-virus antibody has a titre against the homologous strain which is about 100-fold higher than that of the anti-peptide antibodies, only the latter neutralizes across strains (S. Alexander and R.A.L., unpublished). Thus, even during a vigorous immune response against virus, the region represented by these synthetic peptides is not immunogenic; hence, by using peptide immunization one can generate antibody specificities that cannot be obtained in any other way.

Antibody of predetermined specificity in biology

The spread of the use of chemically synthesized peptides to generate antibodies of predetermined specificity is indicated by the number and diversity of experiments recently carried out. The antibodies have been designed for a wide variety of uses.

Detection of proteins predicted from nucleic acid sequences: Anti-peptide antibodies have proved useful in detecting proteins predicted on the basis of nucleic acid sequences to be present in cells. The technology has been particularly successful in discovering DNA and RNA tumour virus proteins implicated in cell transformation. Anti-peptide antibodies have been used to detect the large T antigen of polyoma and SV40 viruses, as well as the cellular transformation-associated protein uniquely expressed in many types of malignant cells[29-31]. Green and Brackmann (personal communication) made an anti-peptide antibody that precipitates the 53,000 molecular weight (53K) protein encoded by the adenovirus EIB transcription unit. As expression of the 53K protein is essential for a fully transformed cell, this antibody together with that made to adenovirus EIA products (see below) should be useful in studying the process of cell transformation. A particularly successful use of anti-peptide antibodies has been in defining the proteins encoded by the oncogenes of the rapidly transforming retroviruses including those of the Moloney sarcoma[32,33], feline sarcoma[34], Rous sarcoma (refs 35, 36 and L. E. Gentry *et al.*, personal communication), avian myeloblastosis[37] and Simian sarcoma virus[38].

Antibodies against functionally active regions of proteins: Peptide hormones are often cleaved from larger precursor proteins, which contain multiple hormones. Anti-peptide antibodies have been used to localize the portions of the 31,000-MW γ-melanocyte-stimulating hormone and 17,500-MW calcitonin precursor which correspond to the functional activities of these two hormones[39,40].

Schaffhauser *et al.* have used anti-peptide antibodies to perturb the functional activity of proteins[41]. The middle T antigen of polyoma virus has been implicated in cell transformation

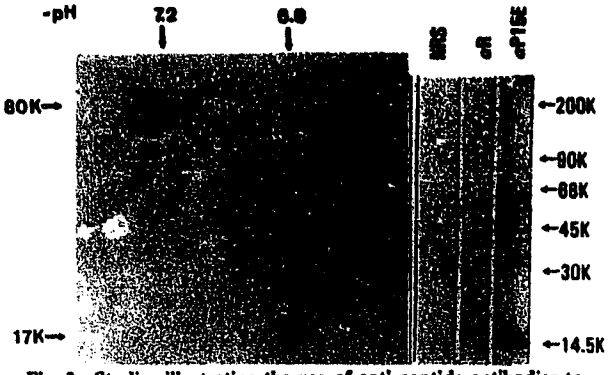


Fig. 3 Studies illustrating the use of anti-peptide antibodies to demonstrate the cleavage of viral protein during virus maturation. Left: immune precipitates of virus infected cells with anti-peptide antibody. Extracts of ^{3}H-leucine-labelled cells were reacted with anti-peptide antibody and the solubilized immune precipitates characterized by two-dimensional gel electrophoresis. The 80K protein is the entire product ($Pr80^{env}$) of the envelope gene and contains N-gp70-15E-C. The 17K protein (pre-15E) was confirmed by radioactive protein sequencing to be the precursor to the 15E anchor protein[44]. Right; immune precipitates of labelled virus showing that amino acids had been cleaved from the C-terminus of pre-15E during virus maturation to yield viral p15E. Virus labelled with ^{35}S-methionine was reacted with normal rabbit sera (NRS); anti-peptide antibody [here called αR to designate that this serum represents antibody against amino acids predicted by right most (3′) possible 15 codons of the viral genome]; and antibody against the mature anchor protein p15E (αP15E). The bulk of the p15E in virus fails to react with αR sera and thus lacks a portion of its C-terminus[44].

and contains a tyrosine kinase activity that predominantly phosphorylates tyrosine 315 of the middle T protein. They synthesized a nonapeptide corresponding to residues 311–319 of the middle T antigen. This antibody not only precipitates middle T in infected cells but inhibits phosphorylation of the protein *in vitro*. Interestingly, the anti-peptide antibody does not react with the middle T antigen when tyrosine 315 is phospho.ylated. Shinnick *et al.*[42] have also used anti-peptide antibodies to localize and perturb the functions of enzymes encoded by viral genomes. The retroviral *pol* gene is about twice as big as necessary to encode the 80,000-MW reverse transcriptase[43]. Gene products were sampled by synthesizing peptides at intervals of about 100 amino acids along the predicted *pol* sequence and antibodies against these peptides used to study *pol* gene products in infected cells. Antibodies to peptides predicted from the 5′ end of the gene precipitated an 80,000-MW protein and inhibited the reverse transcriptase activity whereas antibodies to peptides from the middle of the gene precipitated a 40,000-MW protein and inhibited the virus-associated endonuclease activity. Antibodies to peptides from the 3′ end of the gene precipitate a 20,000-MW protein which according to enzyme inhibition studies seems to be protease. Thus, anti-peptide antibodies are useful not only in detecting the protein product of a gene, but also in identifying its function.

Following protein domains: Anti-peptide antibodies have been used in the difficult problem of following the fate of individual protein domains and have shown that the cleavage and removal of the hydrophilic C-terminus from the retroviral membrane anchor protein takes place during virus maturation[44]. The main envelope glycoprotein, gp70, is anchored into the viral membrane through its attachment by disulphide bonds to the membrane spanning proteins, p15E. Antibody against a synthetic peptide corresponding to the C-terminus of the pre-15E portein was made and used to study the fate of this region of the protein during processing leading to virus formation. In infected cells the antibody detected two proteins of molecular sizes of 80K and 17K corresponding, respectively, to the envelope polyprotein precursor containing gp70 and p15E, and one of the cleavage products (pre-15E) (Fig. 3). However, when the proteins of radioactively labelled virus were studied

with the anti-peptide antibody, it could be seen that the C-terminus predicted from the nucleotide sequence was missing from the mature p15E protein (Fig. 3), thus demonstrating its removal during virus maturation. It remains to be seen if cleavage and processing of C-termini are frequent events which will be found in other systems or if they are peculiar to events involved in viral maturation.

Baron and Baltimore[45] and Semler *et al.*[46] used anti-peptide antibodies to follow poliovirus protein domains, by making antibodies to chemically synthesized peptides corresponding to the genome-linked protein (VPg). Since mature poliovirus proteins are generated by a cascade of cleavages starting with a large precursor (NCVPOO), the peptide of interest is located in various positions in the different intermediate cleavage products, ultimately becoming the C-terminus of a 12,000-MW polypeptide from which 22 amino acids are donated to the 5' end of the genomic RNA. In the Baron and Baltimore experiments antibodies raised against the entire 22 amino acids of VPg as well as a peptide corresponding to its 14 most C-terminal amino acids reacted with the peptide when it was present in five different members of the cleavage cascade. As well as providing the solution of a biological problem, these results demonstrate that the reaction of anti-peptide sera with the native protein is relatively independent of the position of the target in the native structure.

Exon usage: A special example of the use of antibodies of predetermined specificity to follow protein domains is in the study of exon usage during gene expression. Shinnick and Blattner[47] used anti-peptide antibodies to follow exon usage in the immunoglobulin-D system. They synthesized peptides uniquely corresponding to the protein encoded by the exons specific for either the secreted or the membrane-bound form of IgD. Each of the anti-peptide antibodies was shown to be specific for one of the alternative forms of IgD and can now be used to follow the fate of these two proteins in cells. These results, again, demonstrate the production of specific reagents which would be difficult to make by other means. Anti-peptide antibodies have also facilitated the study of exon usage in the adenovirus-2EIA transcription unit. EIA encodes functions which both regulate expression of other early viral genes and have a role in cell transformation[48–51]. The EIA region transcript is processed into at least two overlapping mRNAs (12S and 13S) which share 5' and 3' termini and differ by 138 nucleotides. Since the 12S and 13S mRNAs are in the same reading frame and translation is probably initiated at the common first AUG, it has been assumed that the two proteins encoded by these messages have common N- and C-termini and differ by 46 amino acids unique to the middle of the larger protein[52]. Feldman and Nevins prepared antibody to a 13 amino acid long synthetic peptide predicted from the nucleotide sequence to correspond to a hydrophilic portion of the putative 46 amino acids unique to the larger protein and were able to show that this antibody only reacted with the larger of the two proteins encoded in EIA[53]. This antibody should be very helpful in defining the role of the different EIA proteins in transformation and control of transcription.

Anti-'wrong' reading frame antibodies: We prepared anti-'wrong' reading frame antibodies to study frameshift mutations in viral proteins (A. Sen and R.A.L., unpublished). We used the nucleotide sequence of several sarcoma virus transforming genes to predict the protein sequence which would correspond to +1 and +2 frameshifts far enough to the 3' end of the genome so that premature terminations would not occur. Using these antibodies we were able to detect frameshifted proteins in infected cells. Coupled with transfection of genes, such anti-'wrong' reading frame antibodies may be very useful in comparing the fate of wild-type and mutant proteins in the same cells.

Antibody of predetermined specificity in medicine

Small molecules were first used in the design of synthetic vaccines in 1938 when Goebel made antibody to the carbohydrate antigen diazatinized *p*-aminobenzyl cellobiuronide coupled to horse serum globulin[54,55]. Mice immunized with the preparation acquired active resistance to infection with virulent type III pneumococci[54]. Anderer, working from the results of denaturation and cleavage experiments and assuming that the immunogenicity of the tobacco mosaic virus protein depends on retention of conformation, studied a C-terminal hexapeptide coupled to bovine serum albumin. He showed that antibody to the hexapeptide would precipitate and neutralize the virus[56]. Working with phage, Langbeheim and his colleagues synthesized two peptides from the coat protein of MS-2 and made rabbit antiserum against them[57]. They were able to show binding by one of the anti-peptide antibodies. In their experiment, addition of the rabbit anti-peptide antibody was followed by addition of antisera against rabbit immunoglobulin, thus obscuring whether the anti-peptide antibody had any primary neutralizing activity.

Before synthetic vaccines could be realistically designed to combat eukaryotic viruses, two things were required: a method for obtaining protein sequences of relatively scarce viral proteins, and constraints of the actual or perceived need for conformational determinants had to be overcome. Advances by Sanger and Gilbert and their colleagues in nucleic acid sequencing technology solved the problem of obtaining reliable amino acid sequences of viral proteins, and chemical synthesis of peptides allowed production of antibodies not necessarily restricted, to reactivity with conformational determinants within native proteins. Recently, peptides corresponding to influenza HA1 (refs 25, 58), the hepatitis B surface antigen (HB$_s$Ag)[59–62], the foot-and-mouth disease (FMDV) VP1 protein[63], and the rabies virus glycoprotein[64], have been synthesized. One interesting result in the HB$_s$Ag system is that peptides differing in only two residues were capable of inducing antibodies in rabbits and chimpanzees which were capable of distinguishing the y and d subtypes of HB$_s$Ag[65]. It appears that synthetic peptides can duplicate serological markers known to be of significance from classical virological and epidemiological studies. In the influenza[66] and FMDV systems[63], synthetic peptides induced neutralizing antibodies. Further studies using antibodies of predetermined specificity should, in turn, improve our understanding of the process of virus neutralization and thus guide the design of useful peptides. One can envisage that the reason for strain variation in viral proteins is due to selective pressures of the immune system, and thus that the variable regions signal areas available for, and sensitive to, antibody binding. Alternatively, one can aim for invariant regions with functional activities. An additional theoretical issue important to the design of new vaccines is that an intact protein when free may fold differently from when it is part of a virus particle and thus may not confront the immune system in such a way as to induce neutralizing antibodies. In these cases, synthetic peptides may offer advantages over purified viral capsid proteins (so-called subunit vaccines) because they can induce anti-virus antibodies which are independent of protein folding and can be directed to neutralizing sites on the virus surface.

Practical considerations

Given a nucleotide sequence, the problem often is which peptide to synthesize. As so many different peptides have been shown to induce antibodies reactive with the native protein, the only essential point is to choose one which contains hydrophilic amino acids and is thus likely to be exposed on the surface of the intact molecule. Also, peptides containing hydrophilic groups are likely to be soluble, making their handling and coupling to carrier molecules easier. A number of computer programs have been used to predict secondary structures[67–69], which indicate regions of proteins located on the surface of the molecule and available to antibody but a simple search of the amino acid sequence for charged residues seems to be equally effective for most studies. In the experiments carried out so far, several different carriers and coupling methods have been used with equally good results, and so this choice does not seem

to be critical as long as an immunogenic carrier is used. However, where cultured cells are used, bovine serum albumin should be avoided as a carrier since it is a component of the growth media to which many cellular proteins bind non-specifically and the presence of antibodies to it can confuse immunoprecipitation studies of radioactively labelled cells.

Another question is that of the titre of an anti-peptide antibody compared with that raised by immunization with the intact protein. The answer, as far as we know, is that sometimes it is greater and sometimes less. However an important point is that by using peptide immunization, one can generate antibody specificities which cannot be obtained in any other way.

The minimum size of the peptide chosen is important and should be larger than six amino acids. We generally synthesize peptides of 15 amino acids. Considerably larger peptides have also proved useful[25] but often do not offer any advantage as one risks the problem that they are more likely to assume a fixed conformation distinct from that of the native molecule.

Anti-peptide antibodies can also be used for immunoaffinity purification of rare proteins[70]. The main advantage of anti-peptide antibodies for immunoaffinity purification of proteins is that elution can be accomplished by excess peptide instead of the usual methods of high salt, low *p*H or chaotropic agents. Since the peptide elution is specific for the antigen–antibody union of interest, the recovered protein is less likely to be contaminated with extraneous proteins which were bound to the column at sites other than the antibody combining site. Also, proteins eluted by peptides are more likely to be recovered in a native state.

Theoretical considerations

The reason anti-peptide antibodies so often react with the native structure seems to be that they can adopt a number of conformations in solution, one of which approximates that adopted in the native molecule. If this is the case anti-peptide antibodies should contain a mixture of reactivities to these various conformations with only a small percentage of them actually reacting with the native structure. There are, however, other theoretical possibilities. One is that a protein molecule perturbed by solvent interactions exists as a statistical ensemble of conformative states with similar backbone structures but with variations in side-chain orientations on the surface of the molecule. In this situation the anti-peptide antibody could either 'lock in' a particular conformation, induce a conformation, or, in fact, mould its own combining site which for similar reasons would itself be somewhat sterically mutable. That at least some of the latter notions may be true is supported by our studies on monoclonal antibodies to chemically synthesized peptides (H. Niman and R.A.L., unpublished). We selected monoclonal anti-peptide antibodies against an influenza virus HA1 peptide (amino acids 75–111) and then looked at what percentage would react with the native protein. Surprisingly, 14 of 27 reacted in an enzyme-linked immunoabsorbent assay (ELISA) and immunoprecipitation assays with intact HA1, a number much too large to be compatible with a simple model of occasional shared conformations between the free peptide and its counterpart in the protein. If these results can be extended and generalized, one will have to think in terms of flexible structures in protein, antibody molecules, or both. Alternatively, both in solution and as part of protein molecules, peptides may spend the bulk of their time in a limited number of comparable conformations presumably representing the state of lowest free energy.

Future prospects

The ability to make antibodies to defined regions of proteins will, no doubt, open the way to a number of biochemical experiments on the structure–function relationships of proteins as well as help in the immunological prevention and perhaps treatment of disease. Consideration of the theoretical basis for the immunogenicity of small synthetic peptides has led to questions about the fluidity of regions of protein molecules in solution, as well as the possibility that antibodies induce shape changes in proteins. The somewhat surprising results concerning the immunogenicity of peptides may lead to a better understanding of the dynamics of protein molecules in solution. The fact that such a high percentage of monoclonal anti-peptide antibodies react with the native protein already suggests that something unexpected is on the horizon.

I thank the many investigators who allowed me to cite their experiments before publication. I also thank my colleagues Greg Sutcliffe, Tom Shinnick, Nicola Green, Hannah Alexander, Steve Alexander, Richard Houghton, Jim Bittle, John Gerin, Bob Purcell, Dave Rowlands, Fred Brown, Henry Niman and Art Olson for their collaboration.

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to be critical as long as an immunogenic carrier is used. However, where cultured cells are used, bovine serum albumin should be avoided as a carrier since it is a component of the growth media to which many cellular proteins bind non-specifically and the presence of antibodies to it can confuse immunoprecipitation studies of radioactively labelled cells.

Another question is that of the titre of an anti-peptide antibody compared with that raised by immunization with the intact protein. The answer, as far as we know, is that sometimes it is greater and sometimes less. However an important point is that by using peptide immunization, one can generate antibody specificities which cannot be obtained in any other way.

The minimum size of the peptide chosen is important and should be larger than six amino acids. We generally synthesize peptides of 15 amino acids. Considerably larger peptides have also proved useful[25] but often do not offer any advantage as one risks the problem that they are more likely to assume a fixed conformation distinct from that of the native molecule.

Anti-peptide antibodies can also be used for immunoaffinity purification of rare proteins[70]. The main advantage of anti-peptide antibodies for immunoaffinity purification of proteins is that elution can be accomplished by excess peptide instead of the usual methods of high salt, low *p*H or chaotropic agents. Since the peptide elution is specific for the antigen–antibody union of interest, the recovered protein is less likely to be contaminated with extraneous proteins which were bound to the column at sites other than the antibody combining site. Also, proteins eluted by peptides are more likely to be recovered in a native state.

Theoretical considerations

The reason anti-peptide antibodies so often react with the native structure seems to be that they can adopt a number of conformations in solution, one of which approximates that adopted in the native molecule. If this is the case anti-peptide antibodies should contain a mixture of reactivities to these various conformations with only a small percentage of them actually reacting with the native structure. There are, however, other theoretical possibilities. One is that a protein molecule perturbed by solvent interactions exists as a statistical ensemble of conformative states with similar backbone structures but with variations in side-chain orientations on the surface of the molecule. In this situation the anti-peptide antibody could either 'lock in' a particular conformation, induce a conformation, or, in fact, mould its own combining site which for similar reasons would itself be somewhat sterically mutable. That at least some of the latter notions may be true is supported by our studies on monoclonal antibodies to chemically synthesized peptides (H. Niman and R.A.L., unpublished). We selected monoclonal anti-peptide antibodies against an influenza virus HA1 peptide (amino acids 75–111) and then looked at what percentage would react with the native protein. Surprisingly, 14 of 27 reacted in an enzyme-linked immunoabsorbent assay (ELISA) and immunoprecipitation assays with intact HA1, a number much too large to be compatible with a simple model of occasional shared conformations between the free peptide and its counterpart in the protein. If these results can be extended and generalized, one will have to think in terms of flexible structures in protein, antibody molecules, or both. Alternatively, both in solution and as part of protein molecules, peptides may spend the bulk of their time in a limited number of comparable conformations presumably representing the state of lowest free energy.

Future prospects

The ability to make antibodies to defined regions of proteins will, no doubt, open the way to a number of biochemical experiments on the structure–function relationships of proteins as well as help in the immunological prevention and perhaps treatment of disease. Consideration of the theoretical basis for the immunogenicity of small synthetic peptides has led to questions about the fluidity of regions of protein molecules in solution, as well as the possibility that antibodies induce shape changes in proteins. The somewhat surprising results concerning the immunogenicity of peptides may lead to a better understanding of the dynamics of protein molecules in solution. The fact that such a high percentage of monoclonal anti-peptide antibodies react with the native protein already suggests that something unexpected is on the horizon.

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